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Figure 1

A

TC-A 1 -----CTTTTTCTTTTT-----
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 TC-B 651 GTGTGTTCAACAGTTTTGTTTCTTTTTCTCTTTTCTCTTTCCATCATACATACATACATACATATATATCT-CCGTAGATATGC-
 ACATGC 98
 TC-A 51 GTATATGCGTAAA-AGTGTCTGTCCCAACAAAAATTTTTTTTTTCCGCCTTCCATTTTTTTTTTTTTTGTGTGTTT-
 CCCTTGATCTCTCGAACAG 148
 TC-B 99 GTATATGCGTGAAGAGTGTCTGTCCCAA-----CATTTTTTTTTTTTTTGTGTGTTTCCCTTGATTCC-
 CGAACG 171
 TC-A 149
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 TC-B 172 GGCAGGAAAAAGCTTCTGTTTGACCAAAAAATATAAAATTATTAAGGGCGAGAAAA--GAAA--AAAAAATCAACCG--
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 TC-A 249
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 TC-A 349
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 TC-B 361
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 TC-B 1 MAAVVCFAQFFPPFATPTTFFTCVPLIAGTGCALLFDUAYRIAGTTESEFMDAMPBHQDRAELHAPFESPTCIBHPTAUAARIIVTCOLPHIPD 318
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 TC-B 101 SNAARAAFLQENHDLRLGLCLSPANNRRFNAFLPFLNNAVLORVFXDTGCLNNCGHSEIAAVTAAYETGIVGVPAATYVVPVLDTPAGLVRY 318
 TC-A 132 ARLQSDTHLVNNAHINVFRTDUPVVDLFGDCAUAGGGFFALVPAELVHINISVGLSLQAGLLATPIPPVGVORPULPPIHTVOC 300
 TC-B 132 ARLQSDTHLVNNAHINVFRTDUPVVDLFGDCAUAGGGFFALVPAELVHINISVGLSLQAGLLATPIPPVGVORPULPPIHTVOC 318
 TC-A 201 ARLQSDTHLVNNAHINVFRTDUPVVDLFGDCAUAGGGFFALVPAELVHINISVGLSLQAGLLATPIPPVGVORPULPPIHTVOC 300
 TC-B 201 ARLQSDTHLVNNAHINVFRTDUPVVDLFGDCAUAGGGFFALVPAELVHINISVGLSLQAGLLATPIPPVGVORPULPPIHTVOC 318
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 TC-B 233 VGLTUPPTPAKCTFVUVTPGNCAHIFECGTGCAALATLFGDCLPTGCVVFEJLCELFDAUULQSRRTGIVVPVPTEDARKVHLVVPVITOKAF 418
 TC-A 401 INCFRFLPDVTPFAPRPTLFG 423
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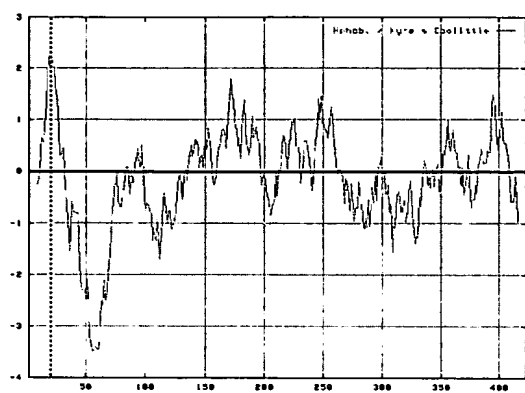
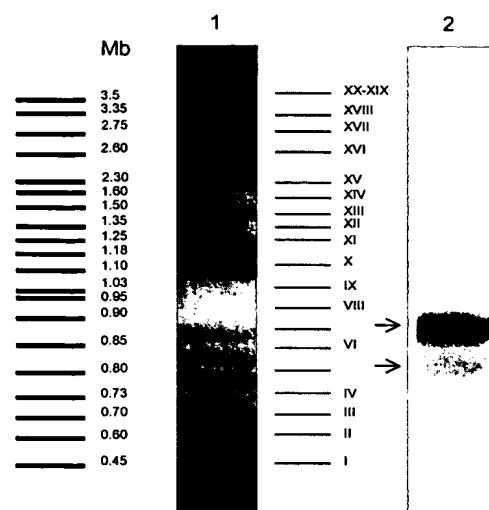
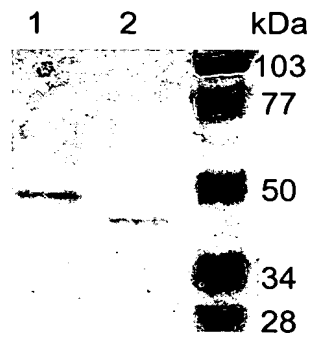
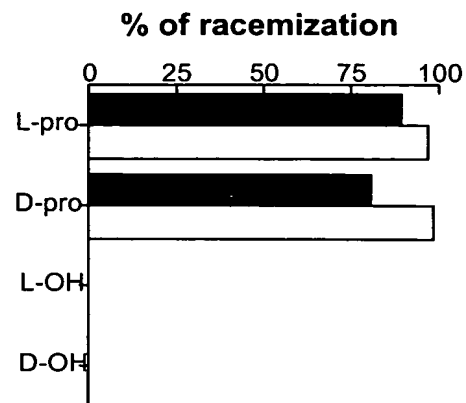
C**D**

Figure 2

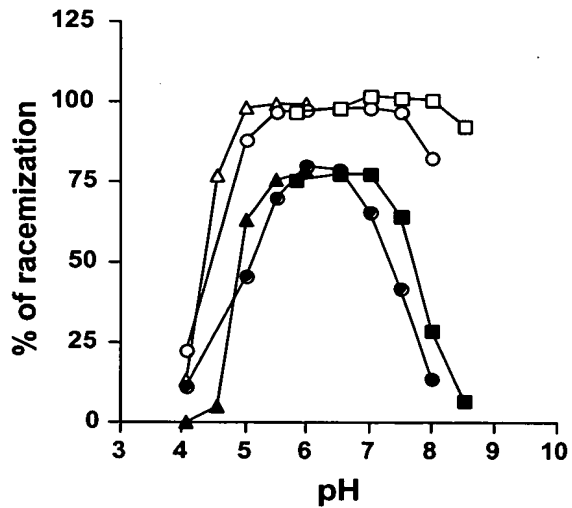
A



B



C



D

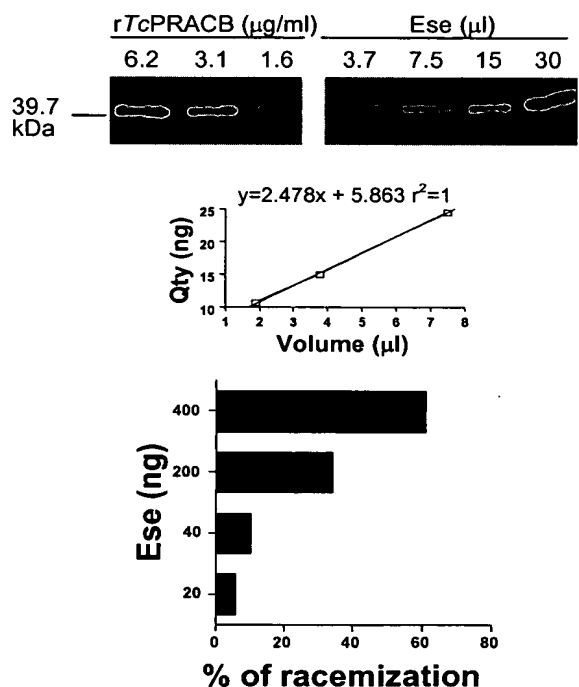


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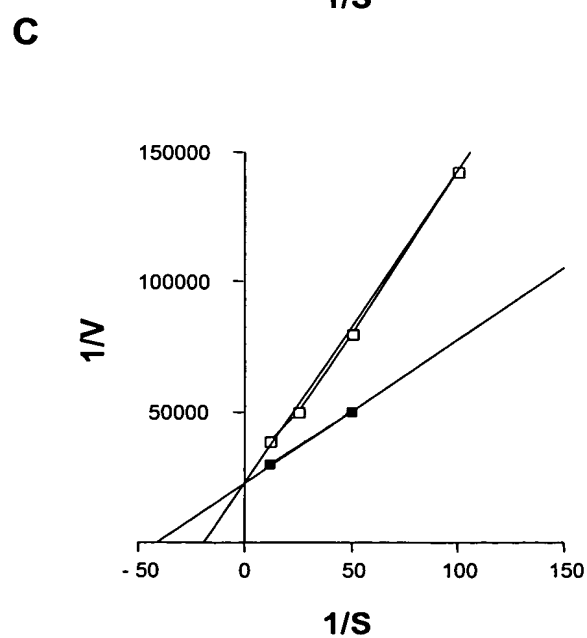
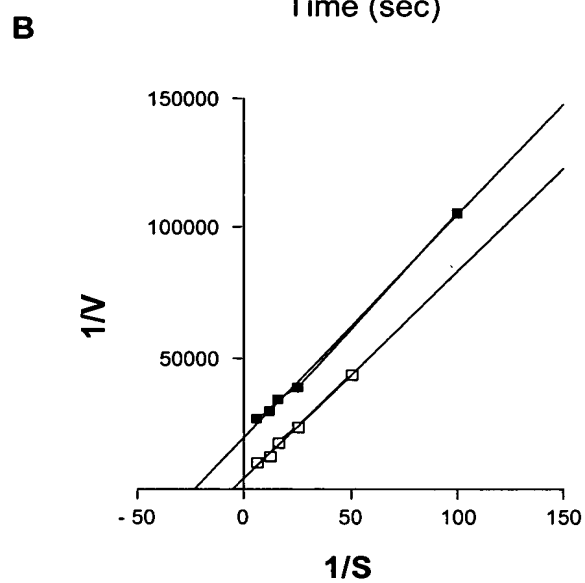
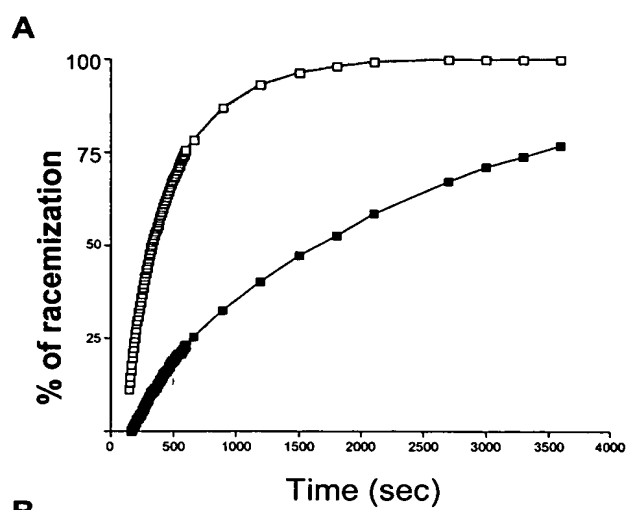


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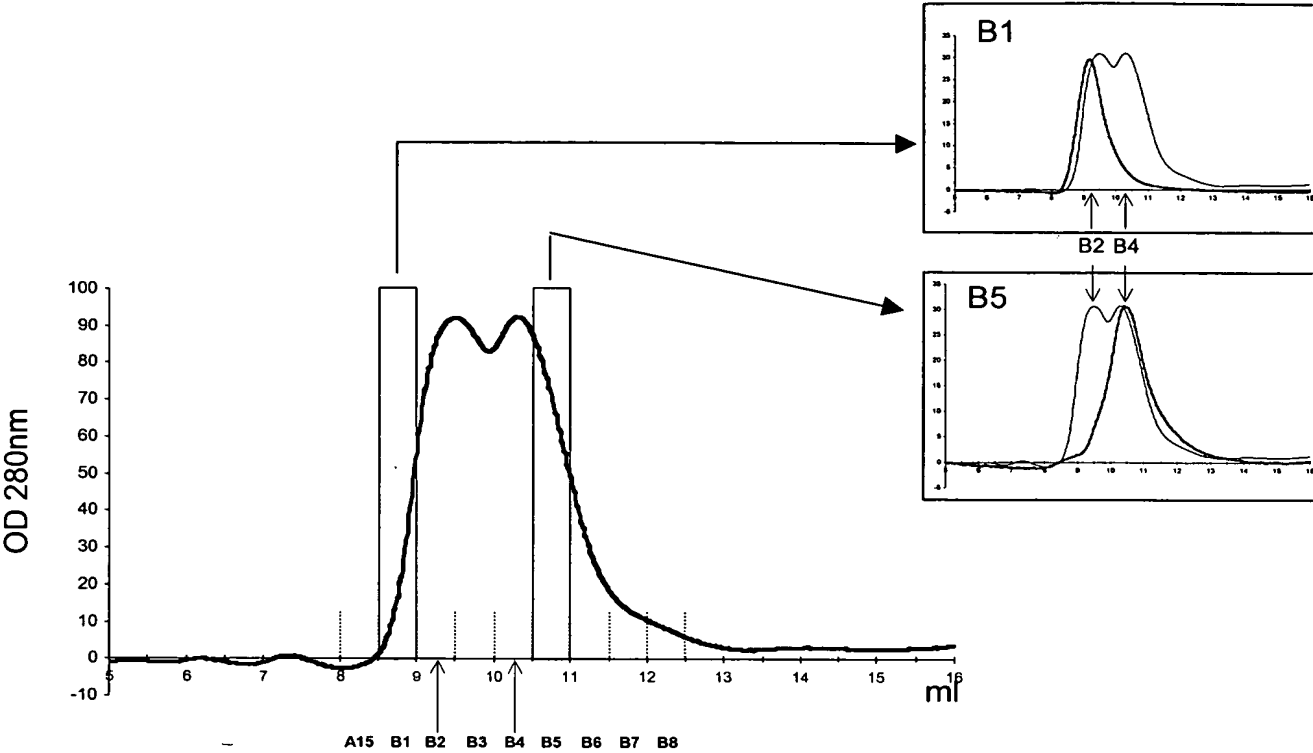


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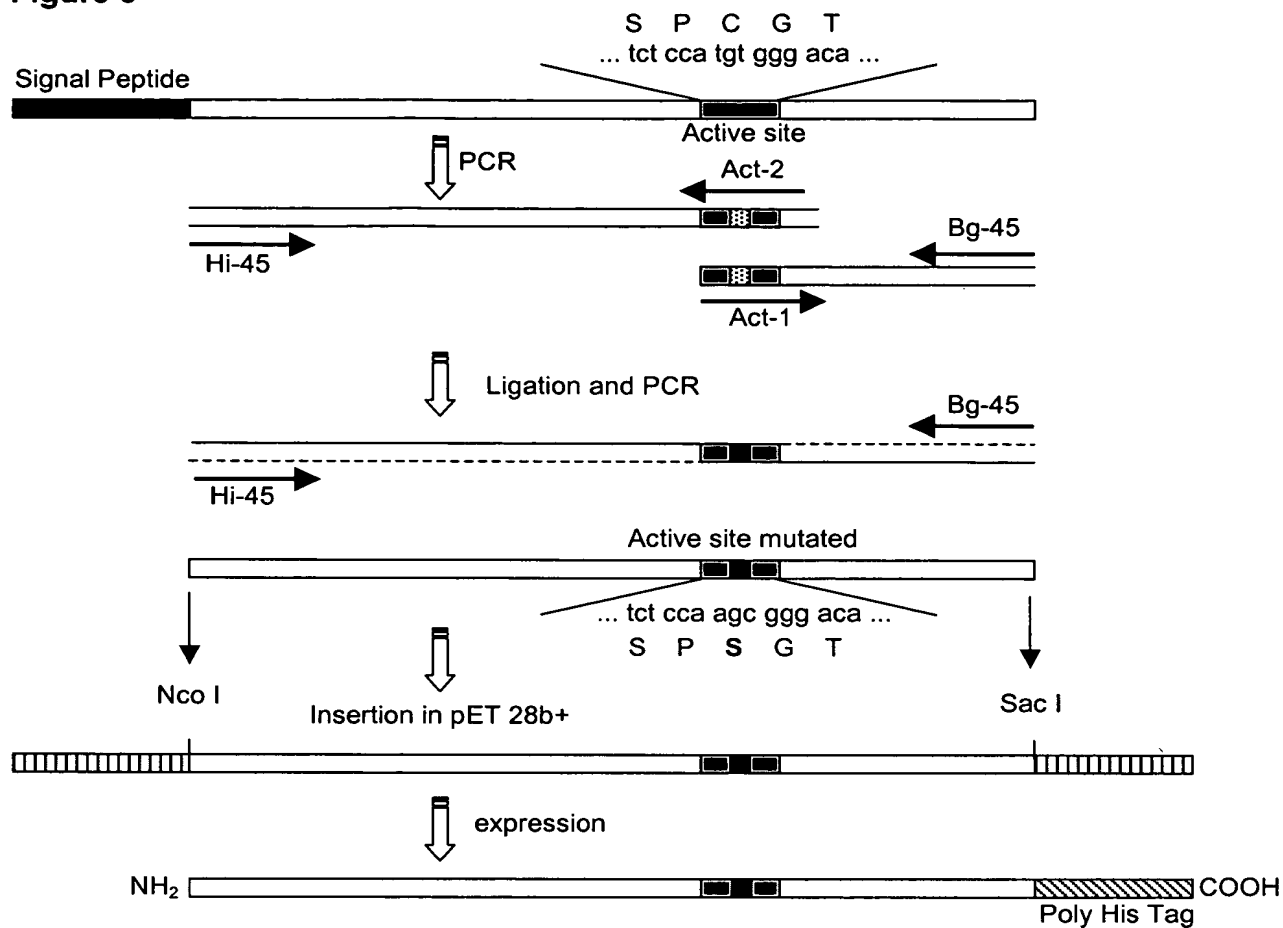


Figure 6

A

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TcPRACB	1	MRKSVCKPKQKFFSAPPFPFFFCVPLIISRTGQKLLFDQYKII	100
C.acklandii	1	MRKSVCKPKQKFFSAPPFPFFFCVPLIISRTGQKLLFDQYKII	100
H.aapiens1	1	MRKSVCKPKQKFFSAPPFPFFFCVPLIISRTGQKLLFDQYKII	100
H.aapiens2	1	MRKSVCKPKQKFFSAPPFPFFFCVPLIISRTGQKLLFDQYKII	100
M.musculus1	1	MRKSVCKPKQKFFSAPPFPFFFCVPLIISRTGQKLLFDQYKII	100
M.musculus2	1	MRKSVCKPKQKFFSAPPFPFFFCVPLIISRTGQKLLFDQYKII	100
R.loti1	1	MRKSVCKPKQKFFSAPPFPFFFCVPLIISRTGQKLLFDQYKII	100
B.melitensis1	1	MRKSVCKPKQKFFSAPPFPFFFCVPLIISRTGQKLLFDQYKII	100
R.meliloti2	1	MRKSVCKPKQKFFSAPPFPFFFCVPLIISRTGQKLLFDQYKII	100
A.cumefaciens3	1	MRKSVCKPKQKFFSAPPFPFFFCVPLIISRTGQKLLFDQYKII	100
X.campestris1	1	MRKSVCKPKQKFFSAPPFPFFFCVPLIISRTGQKLLFDQYKII	100
X.axenopodis1	1	MRKSVCKPKQKFFSAPPFPFFFCVPLIISRTGQKLLFDQYKII	100
P.aeruginosa1	1	MRKSVCKPKQKFFSAPPFPFFFCVPLIISRTGQKLLFDQYKII	100
P.aeruginosa2	1	MRKSVCKPKQKFFSAPPFPFFFCVPLIISRTGQKLLFDQYKII	100
B.melitensis2	1	MRKSVCKPKQKFFSAPPFPFFFCVPLIISRTGQKLLFDQYKII	100
A.cumefaciens2	1	MRKSVCKPKQKFFSAPPFPFFFCVPLIISRTGQKLLFDQYKII	100
R.meliloti1	1	MRKSVCKPKQKFFSAPPFPFFFCVPLIISRTGQKLLFDQYKII	100
R.loti2	1	MRKSVCKPKQKFFSAPPFPFFFCVPLIISRTGQKLLFDQYKII	100
X.axenopodis2	1	MRKSVCKPKQKFFSAPPFPFFFCVPLIISRTGQKLLFDQYKII	100
S.caelicolor1	1	MRKSVCKPKQKFFSAPPFPFFFCVPLIISRTGQKLLFDQYKII	100
A.cumefaciens1	1	MRKSVCKPKQKFFSAPPFPFFFCVPLIISRTGQKLLFDQYKII	100

TcPRACA	101	SNMAEKXVLYQENMDY--LRRLGIMLEPRGHDMGPAFLDPPI	197
TcPRACB	101	SNMAEKXVLYQENMDY--LRRLGIMLEPRGHDMGPAFLDPPI	197
C.acklandii	101	SNMAEKXVLYQENMDY--LRRLGIMLEPRGHDMGPAFLDPPI	197
H.aapiens1	101	SNMAEKXVLYQENMDY--LRRLGIMLEPRGHDMGPAFLDPPI	197
H.aapiens2	101	SNMAEKXVLYQENMDY--LRRLGIMLEPRGHDMGPAFLDPPI	197
M.musculus1	101	SNMAEKXVLYQENMDY--LRRLGIMLEPRGHDMGPAFLDPPI	197
M.musculus2	101	SNMAEKXVLYQENMDY--LRRLGIMLEPRGHDMGPAFLDPPI	197
R.loti1	101	SNMAEKXVLYQENMDY--LRRLGIMLEPRGHDMGPAFLDPPI	197
B.melitensis1	101	SNMAEKXVLYQENMDY--LRRLGIMLEPRGHDMGPAFLDPPI	197
R.meliloti2	101	SNMAEKXVLYQENMDY--LRRLGIMLEPRGHDMGPAFLDPPI	197
A.cumefaciens3	101	SNMAEKXVLYQENMDY--LRRLGIMLEPRGHDMGPAFLDPPI	197
X.campestris1	101	SNMAEKXVLYQENMDY--LRRLGIMLEPRGHDMGPAFLDPPI	197
X.axenopodis1	101	SNMAEKXVLYQENMDY--LRRLGIMLEPRGHDMGPAFLDPPI	197
P.aeruginosa1	101	SNMAEKXVLYQENMDY--LRRLGIMLEPRGHDMGPAFLDPPI	197
P.aeruginosa2	101	SNMAEKXVLYQENMDY--LRRLGIMLEPRGHDMGPAFLDPPI	197
B.melitensis2	101	SNMAEKXVLYQENMDY--LRRLGIMLEPRGHDMGPAFLDPPI	197
A.cumefaciens2	101	SNMAEKXVLYQENMDY--LRRLGIMLEPRGHDMGPAFLDPPI	197
R.meliloti1	101	SNMAEKXVLYQENMDY--LRRLGIMLEPRGHDMGPAFLDPPI	197
R.loti2	101	SNMAEKXVLYQENMDY--LRRLGIMLEPRGHDMGPAFLDPPI	197
X.axenopodis2	101	SNMAEKXVLYQENMDY--LRRLGIMLEPRGHDMGPAFLDPPI	197
S.caelicolor1	101	SNMAEKXVLYQENMDY--LRRLGIMLEPRGHDMGPAFLDPPI	197
A.cumefaciens1	101	SNMAEKXVLYQENMDY--LRRLGIMLEPRGHDMGPAFLDPPI	197

M II

TcPRACA	198	RTGAHLQSGTSESVNASII	298
TcPRACB	198	RTGAHLQSGTSESVNASII	298
C.acklandii	198	RTGAHLQSGTSESVNASII	298
H.aapiens1	198	RTGAHLQSGTSESVNASII	298
H.aapiens2	198	RTGAHLQSGTSESVNASII	298
M.musculus1	198	RTGAHLQSGTSESVNASII	298
M.musculus2	198	RTGAHLQSGTSESVNASII	298
R.loti1	198	RTGAHLQSGTSESVNASII	298
B.melitensis1	198	RTGAHLQSGTSESVNASII	298
R.meliloti2	198	RTGAHLQSGTSESVNASII	298
A.cumefaciens3	198	RTGAHLQSGTSESVNASII	298
X.campestris1	198	RTGAHLQSGTSESVNASII	298
X.axenopodis1	198	RTGAHLQSGTSESVNASII	298
P.aeruginosa1	198	RTGAHLQSGTSESVNASII	298
P.aeruginosa2	198	RTGAHLQSGTSESVNASII	298
B.melitensis2	198	RTGAHLQSGTSESVNASII	298
A.cumefaciens2	198	RTGAHLQSGTSESVNASII	298
R.meliloti1	198	RTGAHLQSGTSESVNASII	298
R.loti2	198	RTGAHLQSGTSESVNASII	298
X.axenopodis2	198	RTGAHLQSGTSESVNASII	298
S.caelicolor1	198	RTGAHLQSGTSESVNASII	298
A.cumefaciens1	198	RTGAHLQSGTSESVNASII	298

M III

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TcPRACB	298	VDCVEIY-GPP-TNPEANYKNVIFGNRQA	394
C.acklandii	298	VDCVEIY-GPP-TNPEANYKNVIFGNRQA	394
H.aapiens1	298	VDCVEIY-GPP-TNPEANYKNVIFGNRQA	394
H.aapiens2	298	VDCVEIY-GPP-TNPEANYKNVIFGNRQA	394
M.musculus1	298	VDCVEIY-GPP-TNPEANYKNVIFGNRQA	394
M.musculus2	298	VDCVEIY-GPP-TNPEANYKNVIFGNRQA	394
R.loti1	298	VDCVEIY-GPP-TNPEANYKNVIFGNRQA	394
B.melitensis1	298	VDCVEIY-GPP-TNPEANYKNVIFGNRQA	394
R.meliloti2	298	VDCVEIY-GPP-TNPEANYKNVIFGNRQA	394
A.cumefaciens3	298	VDCVEIY-GPP-TNPEANYKNVIFGNRQA	394
X.campestris1	298	VDCVEIY-GPP-TNPEANYKNVIFGNRQA	394
X.axenopodis1	298	VDCVEIY-GPP-TNPEANYKNVIFGNRQA	394
P.aeruginosa1	298	VDCVEIY-GPP-TNPEANYKNVIFGNRQA	394
P.aeruginosa2	298	VDCVEIY-GPP-TNPEANYKNVIFGNRQA	394
B.melitensis2	298	VDCVEIY-GPP-TNPEANYKNVIFGNRQA	394
A.cumefaciens2	298	VDCVEIY-GPP-TNPEANYKNVIFGNRQA	394
R.meliloti1	298	VDCVEIY-GPP-TNPEANYKNVIFGNRQA	394
R.loti2	298	VDCVEIY-GPP-TNPEANYKNVIFGNRQA	394
X.axenopodis2	298	VDCVEIY-GPP-TNPEANYKNVIFGNRQA	394
S.caelicolor1	298	VDCVEIY-GPP-TNPEANYKNVIFGNRQA	394
A.cumefaciens1	298	VDCVEIY-GPP-TNPEANYKNVIFGNRQA	394

TcPRACA	395	ITGKAFIMGFNTMLDPDTPDKNGPTLIQ	423
TcPRACB	395	ITGKAFIMGFNTMLDPDTPDKNGPTLIQ	423
C.acklandii	395	ITGKAFIMGFNTMLDPDTPDKNGPTLIQ	423
H.aapiens1	395	ITGKAFIMGFNTMLDPDTPDKNGPTLIQ	423
H.aapiens2	395	ITGKAFIMGFNTMLDPDTPDKNGPTLIQ	423
M.musculus1	395	ITGKAFIMGFNTMLDPDTPDKNGPTLIQ	423
M.musculus2	395	ITGKAFIMGFNTMLDPDTPDKNGPTLIQ	423
R.loti1	395	ITGKAFIMGFNTMLDPDTPDKNGPTLIQ	423
B.melitensis1	395	ITGKAFIMGFNTMLDPDTPDKNGPTLIQ	423
R.meliloti2	395	ITGKAFIMGFNTMLDPDTPDKNGPTLIQ	423
A.cumefaciens3	395	ITGKAFIMGFNTMLDPDTPDKNGPTLIQ	423
X.campestris1	395	ITGKAFIMGFNTMLDPDTPDKNGPTLIQ	423
X.axenopodis1	395	ITGKAFIMGFNTMLDPDTPDKNGPTLIQ	423
P.aeruginosa1	395	ITGKAFIMGFNTMLDPDTPDKNGPTLIQ	423
P.aeruginosa2	395	ITGKAFIMGFNTMLDPDTPDKNGPTLIQ	423
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R.meliloti1	395	ITGKAFIMGFNTMLDPDTPDKNGPTLIQ	423
R.loti2	395	ITGKAFIMGFNTMLDPDTPDKNGPTLIQ	423
X.axenopodis2	395	ITGKAFIMGFNTMLDPDTPDKNGPTLIQ	423
S.caelicolor1	395	ITGKAFIMGFNTMLDPDTPDKNGPTLIQ	423
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Figure 7

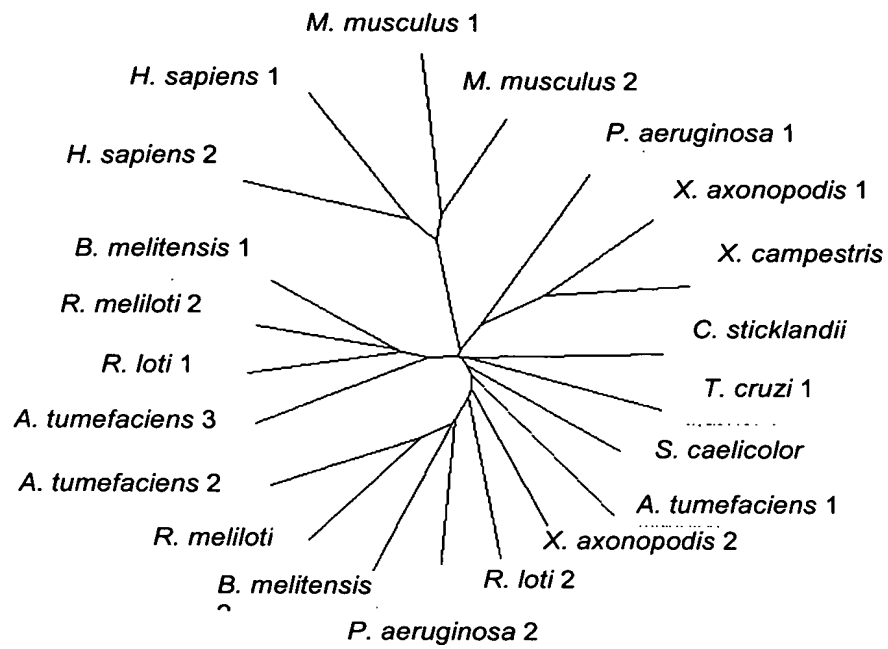


FIGURE 8

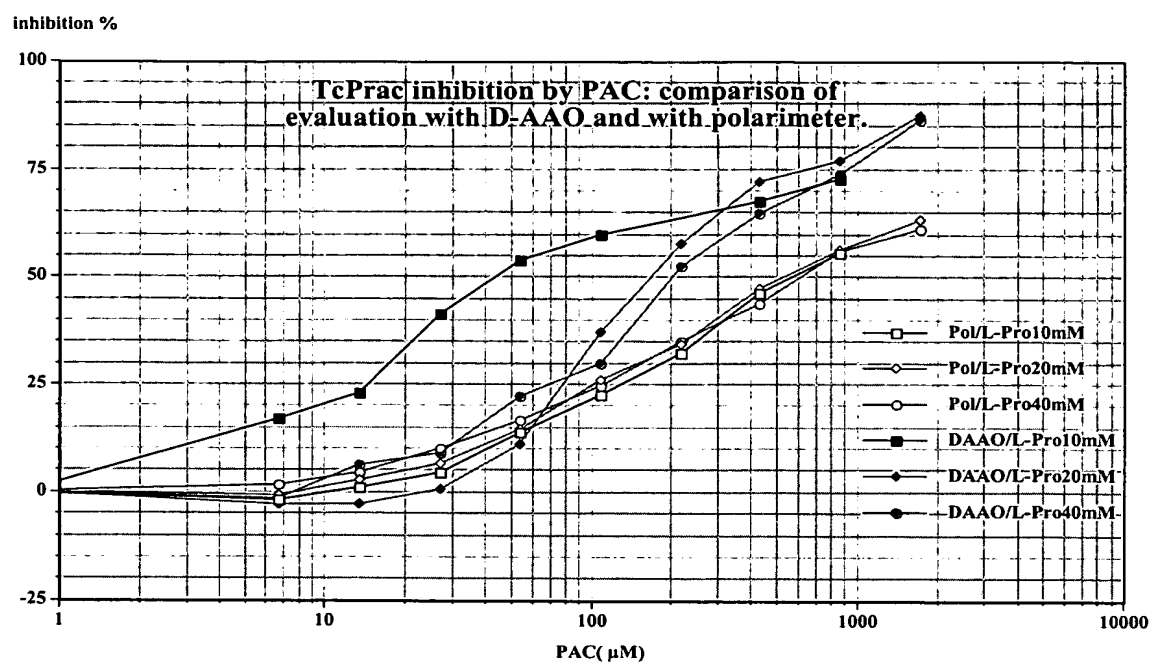


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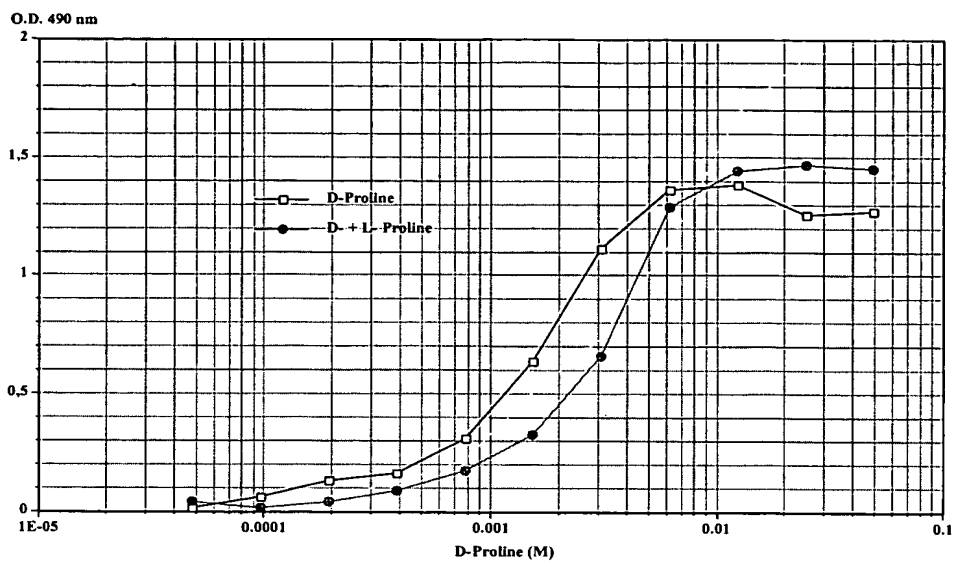


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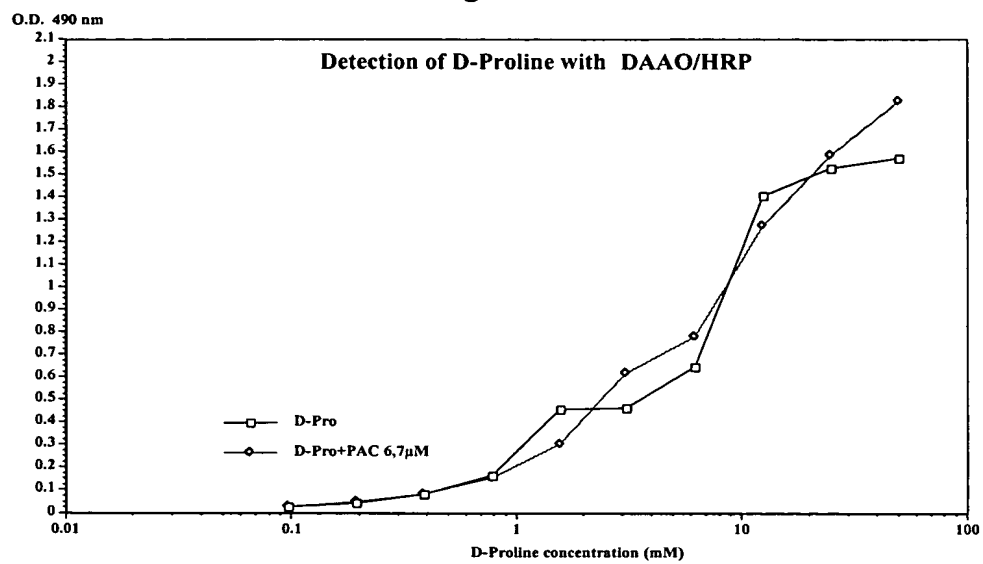


FIGURE 11

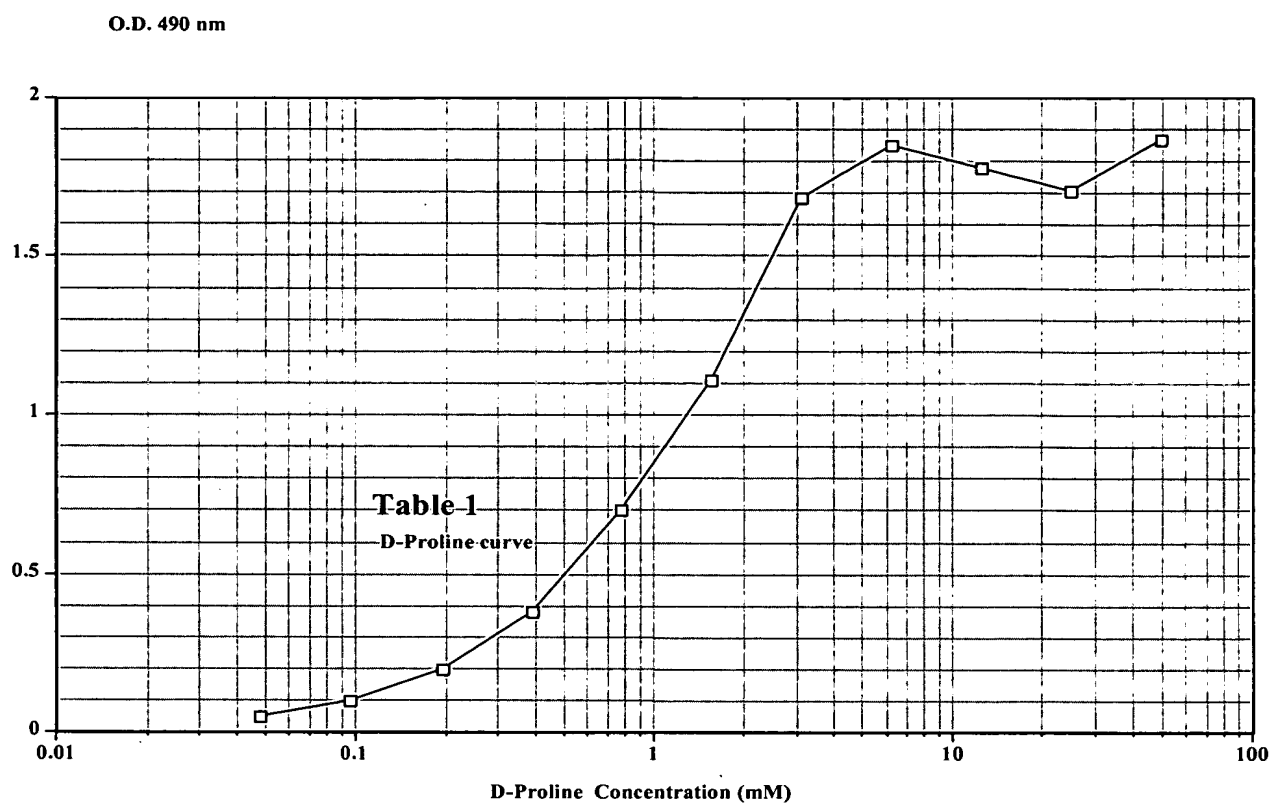


FIGURE 12

The loss of the enzymatic activity of proline racemase after mutagenesis of the Cys160 or the Cys330 residues is shown in the figure below. The results confirm that both residues of a same homodimer unit are implicated in the enzymatic active site of the proline racemase. Furthermore, these results challenge the previous proposed mechanism of proline racemization predicted for the protobacterium *Clostridium sticklandii*, as TcPRAC possesses two active sites per homodimer.

